Crystal Structure of the Receptor-Binding Protein Head Domain from *Lactococcus lactis* Phage bIL170

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Lactococcus lactis, a gram-positive bacterium widely used by the dairy industry, is subject to lytic phage infections. In the first step of infection, phages recognize the host saccharidic receptor using their receptor binding protein (RBP). Here, we report the 2.30-Å-resolution crystal structure of the RBP head domain from phage bIL170. The structure of the head monomer is remarkably close to those of other lactococcal phages, p2 and TP901-1, despite any sequence identity with them. The knowledge of the three-dimensional structures of three RBPs gives a better insight into the module exchanges which have occurred among phages.

Every viral infection starts with the recognition of the host cell through the receptor-binding complex located at the distal part of the virion. Interaction of tailed bacteriophages (*Caudovirales* order) with their bacterial hosts is mediated by a receptor-binding protein (RBP) positioned at the tip of the tail. Interestingly, a structural similarity was recently found between the recognition domain of the RBP from a lactococcal bacteriophage and those of adenoviruses and reoviruses, which invade mammalian cells (18). These data suggested that evolutionarily distant viruses might have a common ancestral gene, despite a lack of sequence similarity.

Virulent lactococcal bacteriophages (infecting *Lactococcus lactis*) are a significant threat to the dairy industry. Hundreds of phages infecting various *L. lactis* strains have been isolated worldwide (11). Most lactococcal phages disturbing fermentations belong to one of three phage groups, namely, 936, c2, and P335, of the *Siphoviridae* family (10, 12). Members of the 936- and P335-like phage groups recognize their host through an interaction between their RBP (7) and unknown saccharidic receptors at the host cell surface (17, 18, 21).

We previously determined the crystal structures of two RBPs, from the virulent lactococcal phage p2 (936 group) (18, 21) and the temperate phage TP901-1 (P335 group) (17). Despite amino acid sequence diversity and infecting different *L. lactis* strains, the RBPs of these two phages are closely related and are formed of three monomers related by a threefold noncrystallographic axis. Each monomer comprises three domains: the N terminus (named shoulders), the interlaced neck, and the head domain at the C terminus. We have suggested that this last domain harbors a putative saccharide binding site and recognizes the host receptor (5, 9, 17, 18, 21).

The complete genome of bacteriophage bIL170 is available (4),

and the gene coding for its RBP was recently identified (6). The deduced RBPs from phages p2 and bIL170 exhibit an overall 52% sequence identity for 267 residues. However, the first 134 residues, which correspond to phage p2 shoulders, share 89% identity, while the remaining 133 residues (the neck and head domains in the RBP of phage p2) share only 15% identity. The diversity in these host recognition domains is in agreement with their different host ranges. The lack of structural knowledge due to the absence of sequence identity prompted us to express and crystallize the RBP head domain of phage bIL170.

bIL170 head domain production and characterization. *orf20*, coding for the RBP of phage bIL170, was cloned in the Gateway pDEST14 vector (25) with a C-terminal His$_6$ tag, and the resulting vector was transformed in *E. coli* Rosetta-pLysS strains. The head domain was cloned between residues Asn 155 and Leu 267 (Fig. 1A) using the same procedure. With both constructs, cell growth and lysis as well as protein purification were performed as previously described (17, 23, 24). Both constructs led to expression of soluble proteins, but the full-length RBP was not stable with time, in contrast to the head domain, which was subjected to binding studies and crystallization assays.

Saccharides bind to bIL170 head domain. We have used tryptophan fluorescence quenching to measure saccharide binding to the RBP of lactococcal phage p2 and to its head domain (21). With the bIL170 head domain, however, tryptophan quenching experiments remained unsuccessful. Tyrosine fluorescence, although weaker than that of tryptophan, was therefore investigated. Fluorescence quenching experiments were carried out on a Perkin-Elmer LS 50B spectrofluorimeter according to procedures described previously (17, 21) by recording the quenching of the intrinsic tyrosine fluorescence upon addition of sugar aliquots. The fluorescence signal exited at 280 nm and read at 346 nm could be significantly quenched by sugars. The values obtained (Table 1) are similar to those found with both the full-length RBP of phage p2 and its head domain (Table 1) (21). Therefore, the head domain of the bIL170 RBP binds saccharides as efficiently as the RBP of phage p2. Taken altogether, these

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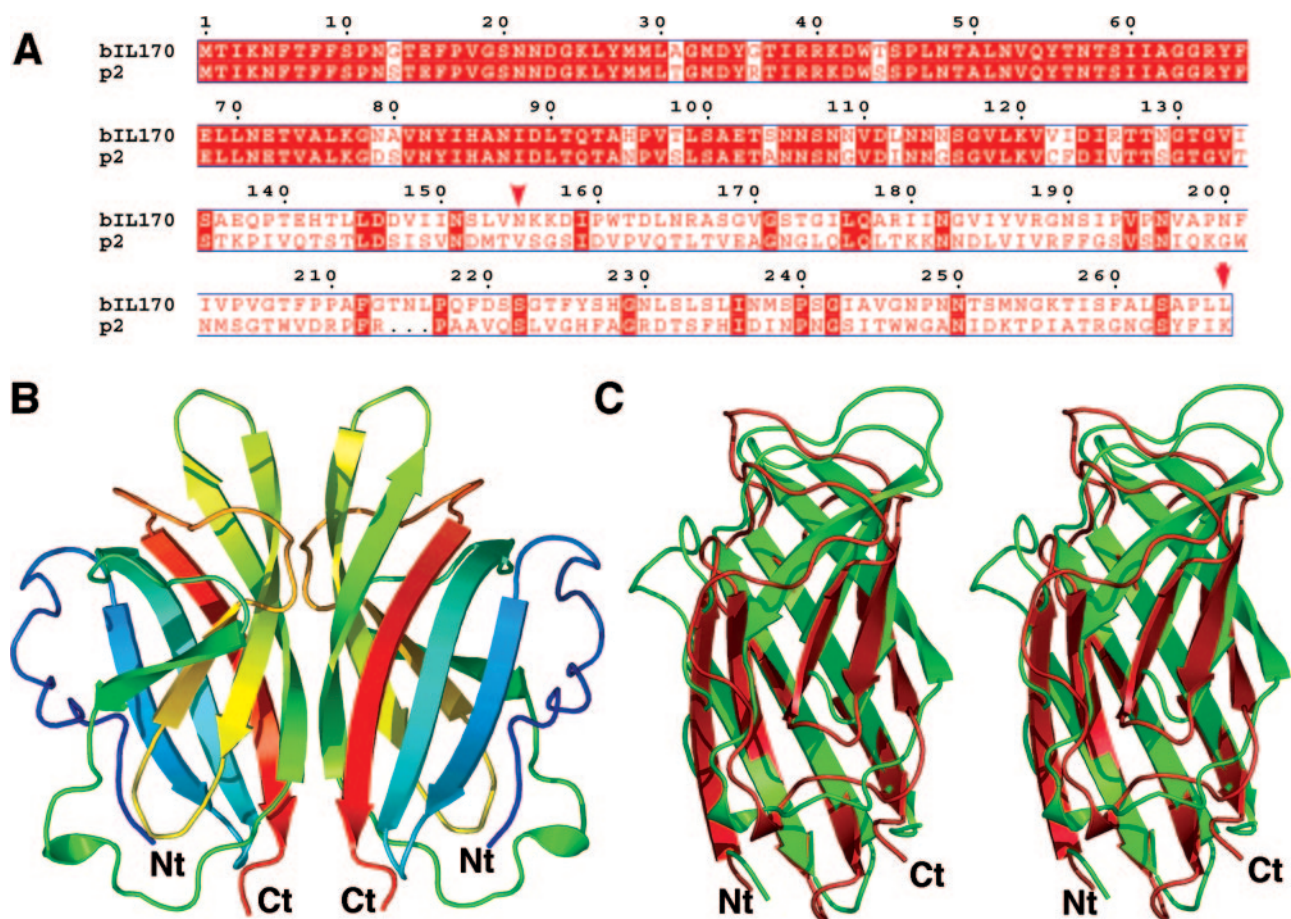
FIG. 1. Compared sequence and structure of the RBPs from lactococcal phages bIL170 and p2. Panel A, amino acid sequence alignment of the RBP from phages bIL170 and p2 (alignment made by MULTALIN [http://prodes.toulouse.inra.fr/multalin/multalin.html]). Red shading highlights conserved amino acids. Two red arrows indicate the cloned head domain. Panel B, view of the bIL170 RBP dimer as found in the crystal. The twofold axis is vertical. Colors vary from blue to red, from N to C terminus (rainbow coloring scheme). Panel C, ribbon stereo view of the bIL170 RBP monomer (green) superimposed on the p2 RBP monomer (red). The views were made with Pymol (http://www.pymol.org).

results indicate that the bIL170 RBP head is functional in solution, like the head of p2 RBP, and that saccharides bind near a tyrosine residue.

Overall crystal structure of bIL170 head domain. Crystallization trials were performed with sitting drops at 291 K using a nanodrop-dispensing robot (Honeybee 961; Cartesian, Inc.) (19). Crystals of selenomethionine-substituted head domain were observed by using the Structure Screen kit with condition

TABLE 1. K_d constants for three saccharides, obtained by fluorescence quenching

RBP or head domain (reference)	K_d value (nM)[a]		
	Glycerol	Galactose	N-acetyl muramic dipeptide
RBP head of bIL170 (this work)	190 ± 40	110 ± 40	100 ± 30
RBP of p2 (21)	260 ± 50	170 ± 30	120 ± 30
RBP head of p2 (21)	180 ± 40	160 ± 30	140 ± 30

[a] Results are expressed as means ± standard deviations.

A9 and with the protein concentration at 7 mg/ml. Data were collected at the European Synchrotron Radiation Facility (lines ID14-3 and ID 23-1) and were processed using MOSFLM/SCALA (3). Crystals belong to space group P3₁21, with cell dimensions $a = b = 69.65$ Å and $c = 95.35$ Å. Two monomers in the asymmetric unit led to a Vm value of 2.79 Å³/Da and 55.6% water in the crystal. Phases were calculated with SHELX (16) and extended to 2.3 Å with RESOLVE (20). A partial model was used as an input for ARP/WARP (13), which yielded the complete model, and then refined using REFMAC5 (14). The final R and R-free values at a 2.3-Å resolution are 0.242 and 0.268, respectively. No residues are found in the PROCHECK generously or disallowed regions (8).

The monomer has a quasidouble Greek key fold close to those found in p2 and TP901-1 RBPs (Fig. 1). After superposition with the p2 RBP head monomer, a root-mean-square deviation (rmsd) of 2.17 Å calculated for 96 residues of 110 was measured. Applying the same procedure to the RBP head of the lactococcal phage TP901-1 yielded an rmsd of 2.10 Å calculated for 94 residues of 110. Between the RBP heads of p2
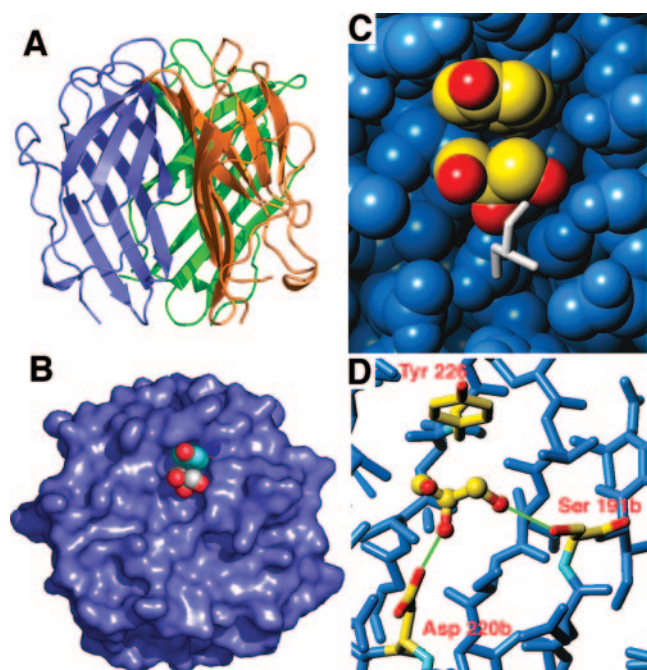
FIG. 2. Three-dimensional structure of the trimer model of the bIL170 RBP head. Panel A, ribbon view of the trimer assembly. Colors are blue, orange, and green for monomers A, B, and C, respectively. Panel B, view of the overall molecular surface of the head domain trimer with tyrosine 226 displayed (blue and red spheres) as well as a modeled glycerol molecule (white and red spheres). Panel C, view in sphere representation of the binding crevice. The glycerol molecule in the position observed in the RBP structure of another lactococcal phage of the 936 group (phage p2) is represented as white sticks. A glycerol molecule has been modeled in the site, close to Tyr 226 (protein surface in blue; Tyr 226 and glycerol are colored yellow and red). Panel D, representation of the modeled glycerol molecule in its binding site (protein in blue; glycerol, Tyr 226, Ser 191b, and Asp 220b are colored in atom mode: C, yellow; O, red; N, blue). The putative hydrogen bonds are displayed in green (views made with Turbo-Frodo [15]).

and TP901-1, an rmsd value of 0.80 Å was obtained with 96 residues of 100. A dimer was present in the crystal, however (Fig. 1), and not a trimer as for the two other RBPs.

The trimeric model. It can be postulated, however, that the bIL170 RBP head is a homotrimer on the basis of several arguments. First, the amino acid identity between the shoulder domains of the p2 and bIL170 RBPs is 89% (Fig. 1A), which ensures an identical assembly for both domains. Second, the head domain of the p2 RBP alone has been revealed to be a trimer (18). Finally, the head domain of the bIL170 RBP is functional in solution. It was therefore a surprise when the X-ray structure revealed the presence of a dimer in the crystal. The molecular surface area covered upon dimerization is quite low, however, being only 490 Å^2, which represents 9.2% of the whole monomer surface. It is therefore very likely that the bIL170 RBP head is a weak dimer and is the result of a crystallization artifact. We could superimpose three monomeric bIL170 RBP head domains onto the p2 RBP trimer. The resulting trimer (Fig. 2A and B) does not exhibit steric clashes involving main chains. Examination of the surface of the trimer revealed the presence of a crevice located at the interface between two monomers (Fig. 2B and C). Such crevices between monomers have been also observed in the RBPs of lactococcal phages p2 and TP901-1.

The putative saccharide-binding site. Interestingly, a Tyr 226 side chain forms the upper wall of the crevice between monomers (Fig. 2D). This tyrosine is the only one to be largely accessible to a solvent in the RBP head domain and is therefore the one titrated. Interestingly, superposition of the p2 RBP head domain onto the bIL170 RBP head trimer brings the bound glycerol (21) in the crevice of the modeled trimer (Fig. 3A). A glycerol molecule has been fitted in the crevice of the modeled trimer, in stacking interaction with Tyr 226 (Fig.
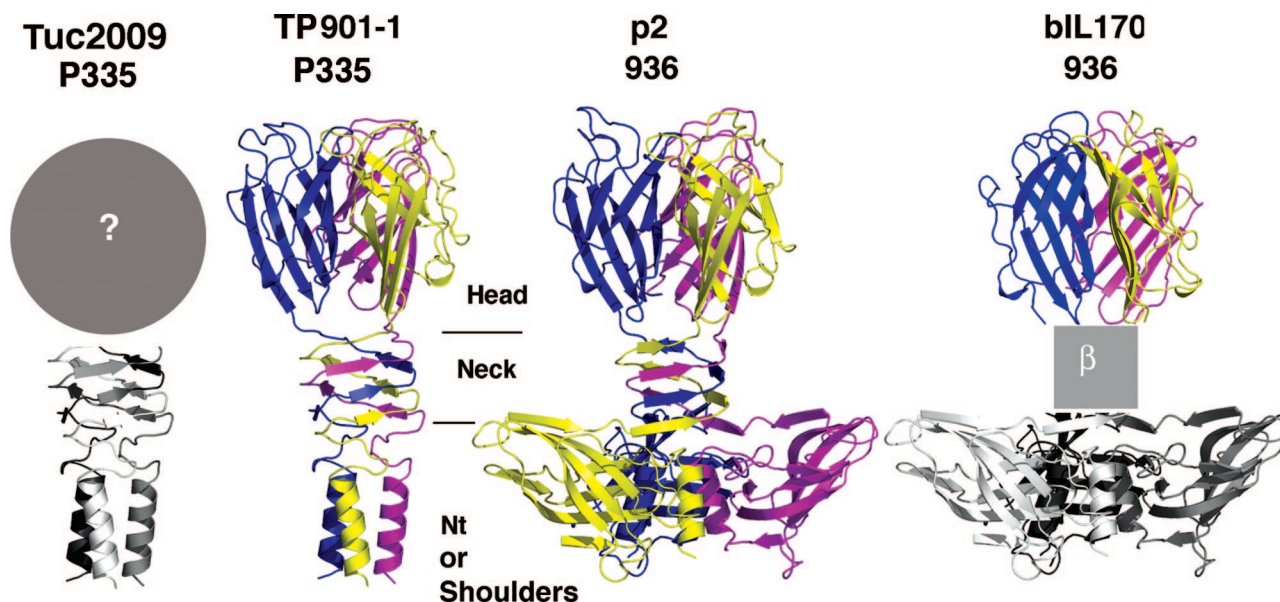

FIG. 3. Comparison of the RBP atomic models from four phages. Two phage RBPs are from the 936 group, namely, p2 (18) and bIL170 (this work), while the two other phages are from the P335 group, namely, TP901-1 (17) and Tuc2009 (model). RBPs are represented on the same scale, and their three domains, N-terminal (or shoulders in p2), neck, and head, have been labeled Nt, Neck, and Head, respectively. The experimentally determined structures are represented in ribbons colored by chain, blue, pink, and yellow. The analogous modeled structures are represented in gray ribbons. Gray squares or circles represent the still-unknown structures.

3) (17, 21). Besides this interaction with Tyr 226b, hydrogen bonds could be established with Ser 191b and Asp 220a. Other residues, in contact or near the glycerol molecule, are half aliphatic and half polar noncharged. This mix of residues is typical of saccharide binding sites, such as those observed in lectins (1, 2).

Comparisons with other phage RBPs. The crystal structure of the host recognition domain of the phage bIL170 is the third structure of an RBP or RBP component to be solved for lactococcal phages and also for phages infecting gram-positive bacteria (Fig. 3). However, thanks to sequence similarity searches, three-dimensional structures of the RBP heads of other phages can now be inferred from sequence comparisons (Fig. 3). Sequence analysis of the three structurally determined RBP heads (using BLAST) against the NR database returned 11, 12, or 5 similar domains of lactococcal phage RBPs, using the p2, TP901-1, or bIL170 sequence as a query, respectively. This result thus extends the space of structurally known head domains significantly. Interestingly, the triple β-barrel fold crosses the border between phages of the 936 and P335 groups in both directions.

As mentioned above, the search for sequences similar to the bIL170 head domain returned five lactococcal phage RBPs, those of phage bIL66 (97% identity; E = 1e−56), phage P008 (95% identity; E = 1e−55), phage P113G/p272 (94% identity; E = 2e−54), phage bIL309 (48% identity; E = 2e−22), and phage bIL286 (47% identity; E = 1e−21). The first three phages are virulent and belong to the 936 group (as bIL170). Their polypeptidic chains upstream of the head domains are all 159 residues long. Due to the very high sequence identity, this chain (containing the shoulders and neck domains) should share a fold (β-barrel) nearly identical to those of phages bIL170 and p2. In contrast, the two latter phages, which are *L. lactis* prophages and belong to the P335 group (as TP901-1), present a strikingly different picture. The polypeptidic chains upstream of the RBP head domains of prophages bIL309 and bIL286 are 1,333 and 703 residues long, respectively. Furthermore, while in the three former RBP shoulders the folds are formed of β-strands, in the two latter RBPs several G-X-X-G repeat motifs are observed, a hallmark of parallel triple helical structures. A similar kind of triple-helix bundle has been observed at the N terminus of phage TP901-1, also a P335 temperate phage, but with a smaller size (30 residues) (17). Recently, the gene coding for the RBP of phage TP901-1 was successfully exchanged with the analogous gene of the temperate phage Tuc2009 (P335 group). The chimerical TP901-1 phage infected the Tuc2009 host strain efficiently and thus displayed an altered host range compared to that of the wild-type phage TP901-1 (22). Molecular biology data indicated that the temperate phage Tuc2009 has an RBP N terminus similar to that found in TP901-1 (22).

To date, with the small number of structures solved and the structural elements inferred from analysis of sequence in a structural context, it seems that virulent phages of the 936 group possess shoulders formed of an assembly of three β-barrels, while phages from the P335 group possess a triple-helical N terminus (Fig. 3). These N-terminal domains, however, display a large variability in size, ranging between ~60 and ~1,300 residues. This difference in the length of the RBP domain anchoring the molecule to the phage baseplate should have a profound influence on the overall structure located at the distal part of the tail.

Our results demonstrate that the fold repertoire of RBP heads is smaller than what is suggested by sequence comparisons. With no exception to date, this fold is shared between RBPs of lactoccocal phages and their functional homologues in reo- and adenoviruses. Interestingly, functional behavior, as depicted by sugar binding, can be closely related between RBP heads displaying no sequence diversity.

Protein structure accession number. Coordinates and structure factors have been deposited with the Protein Data Bank as entry 2FSD.

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